Materials Letters 168 (2016) 116–120

Contents lists available at ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/matlet

Fabrication of cell-penetrable microfibrous matrices with a highly porous structure using a simple fluidic device for tissue engineering

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ARTICLE INFO

Article history:
Received 19 August 2015
Received in revised form
4 January 2016
Accepted 10 January 2016
Available online 11 January 2016

Keywords:
Biomimetic
Microfiber
Porous materials
Scaffold
Cell penetration

ABSTRACT

Poly(ε-caprolactone) (PCL) microfibrous matrices with a highly porous structure were fabricated using a simple fluidic device and precipitation method. Organic solution (dichloromethane) containing PCL and ethanol were used as the discontinuous and continuous phases, respectively, in the fluidic device. The PCL solution was precipitated at the tip of the needle upon the contact with the ethanol, forming uniform fibers. The precipitated fibers were collected at the bottom of a vial containing ethanol. The increase in the flow rate of the continuous phase led to the decreases in the diameter of microfiber and pore size of the matrix. Three-dimensional confocal microscopy images confirmed the highly porous structure of the microfibrous matrices that is sufficient for cell penetration. A cell proliferation assay revealed a faster rate of cell proliferation on the microfibrous matrix compared with the typical electrospun-fibrous matrix. These results suggest that the microfibrous matrix could potentially be used for tissue engineering.

1. Introduction

Due to an extracellular matrix (ECM)-like structure, fibrous matrices have attracted much attention regarding the regeneration of damaged tissues and especially two-dimensional tissues like the periosteum, muscle fascia, and skin [1,2]. Many researchers have used electrospinning to demonstrate the fabrication of two-dimensional porous matrices and three-dimensional tubular scaffolds from various synthetic and natural polymers [3,4]. However, the small pore size of an electrospun-fibrous matrix limited the cell penetration and growth inside the matrix due to the typical dimensions of cells, resulting in the proliferation at the surface of the matrix [5,6].

Several research groups have tried to fabricate a fibrous matrix with a highly porous structure to provide a large surface area and facilitate cell penetration. In an effort to increase the pore size, Kim et al. electrospun poly(ε-caprolactone) (PCL) solution containing a blowing agent (azodicarbonamide) and then decomposed the blowing agent to generate micropores [7]. Although the average pore size was slightly increased, the micropores were not well interconnected, resulting in cell proliferation on only the top surface of the matrix. Lee et al. ultrasonicated an electrospun nanofibrous matrix to enlarge both the porosity and pore size, and consequently demonstrated cell penetration in the matrix [8]. However, the average pore size was still less than 15 μm and the tensile strength decreased after ultrasonication.

Previously, we developed a simple fluidic device that was fabricated with a Tygon® tube, a syringe needle, and a glass capillary tube for the production of uniform microspheres [9]. By modifying the technique, we fabricated a highly porous matrix with a uniform fiber diameter from PCL. The highly porous and microfibrous matrices facilitated cell penetration and proliferation inside the matrices. We believe that this highly porous matrix with an ECM-like structure can be used as a scaffold for the regeneration of various two-dimensional tissues in the human body.

2. Materials and methods

2.1. Fabrication of highly porous matrices

Microfibrous matrices were fabricated using a simple fluidic device with two flow channels, consisting of a syringe needle (30 g), a silica capillary tube (10 μm i.d. Polymicro Technologies), a glass capillary (Aceglass), and a Tygon® tube (1/16 in. i.d. × 3/16 in. o.d.) [9]. A silica capillary tube with a length of 1 cm was inserted onto the syringe needle, which was fixed with epoxy adhesive. The simple fluidic device was fabricated by inserting the

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http://dx.doi.org/10.1016/j.matlet.2016.01.045
0167-577X/

syringe needle with the silica capillary tube and then glass capillary tube into the Tygon® tube, which was fixed with epoxy adhesive (Fig. S1). PCL (M_w≅80,000, Sigma-Aldrich) was used as a polymer for fibrous matrices. PCL solution in dichloromethane (DCM, Sigma-Aldrich) was used as the discontinuous phase in the fluidic device, where ethanol served as the continuous phase. The discontinuous and continuous phases were introduced by syringe pumps (NE-1000, New Era Pump Systems Inc.) at the independently adjusted flow rates. The flow rate of the continuous phase was varied from 0.5 to 3.0 mL/min, while the flow rate of the discontinuous phase was set at 0.02 mL/min. The precipitated PCL fiber was collected in the collection phase (ethanol) under gentle stirring using an orbital shaker. A microfibrous PCL matrix was obtained after washing with ethanol three times and then drying at room temperature. The fiber formation in the glass capillary was observed by digital microscopy (Dino-Lite, Torrance). Scanning electron microscopy (SEM, S-4800) was used to characterize morphologies of the fiber and microfibrous matrix. The pore size of the resultant microfibrous matrix was measured by converting SEM images to binary and thresholding images using ImageJ 'Analyse Particles' feature [10].

2.2. Cell culture

The microfibrous matrix was sterilized by immersion in 70% ethanol for 24 h, washed with PBS (Invitrogen) five times, and stored in a culture medium for 1 day before cell seeding. For comparison, an electrospun-fibrous matrix (Fig. S2) was prepared from PCL using a typical electrospinning setup [11]. GFP-expressing NIH-3T3 (New England Biolabs) was used as a model cell. Cell dispersion (1×10^7 cells/20 mL culture media) was dropped onto the top of the each fibrous matrix immersed in culture media (Fig. S3). After 4 h, the cell-seeded fibrous matrix was gently washed with culture media to remove any unattached cells. The GFP-expressing NIH-3T3 cells were maintained in an α-minimum essential medium (α-MEM, without L-ascorbic acid, Invitrogen) supplemented with 10% FBS and 1% antibiotics (containing penicillin and streptomycin, Invitrogen). The cultures were maintained in an incubator at 37 °C in a humidified atmosphere containing 5% CO_2 and the media were changed every other day. The cell proliferation was evaluated usin.g the cell counting KIT-8 (Dojindo Laboratories). Confocal microscopy (LSM710, Carl Zeiss) was used to observe the cells on the fibrous matrix.

3. Results and discussion

The silica capillary tube with a length of 1 cm was inserted onto the syringe needle to reduce the diameter for the discontinuous flow, which was fixed with epoxy adhesive. The simple fluidic device with two-way flow channels was fabricated by inserting the needle with the silica capillary and then glass capillary into the Tygon® tube, as shown in Fig. S1 [9]. Fig. 1 show a schematic diagram of the simple fluidic device for the fabrication of microfibrous matrices and photograph images of a glass capillary at different flow rates of the continuous phase. The PCL solution in DCM (discontinuous phase) was introduced into the fluidic device through the silica capillary, contacted the ethanol (continuous phase), precipitated at the tip of the silica capillary, and flowed along the glass capillary into a 10-mL vial containing ethanol (collection phase). The precipitated PCL microfibers were stacked on the bottom of the vial. The resultant microfibrous matrices were gently washed with excess ethanol three times and harvested from the vial, followed by drying at room temperature.

Fig. 2 show SEM images and the variations in the diameter of the fiber and pore size of the microfibrous matrix prepared at the different flow rates of the continuous phase. The increase in the flow rate of the continuous phase from 0.5 to 3.0 mL/min led to the reductions in fiber diameter from 14.9 to 2.3 μm and pore size from 54.6 to 25.1 μm. The reduction in the fiber diameter was attributed to the increased shear stress imposed onto the discontinuous phase. In addition, the fibers with a smaller diameter would have low mechanical strength and thus be closely stacked in the vial containing ethanol, which is attributed to the reduction in pore size. Typically, electrospun nanofibrous matrices have a fiber diameter of less than 1 μm and a pore size of less than 10 μm [12,13]. For comparison, an electrospun-fibrous matrix was prepared using a typical electrospinning setup. The electrospun-

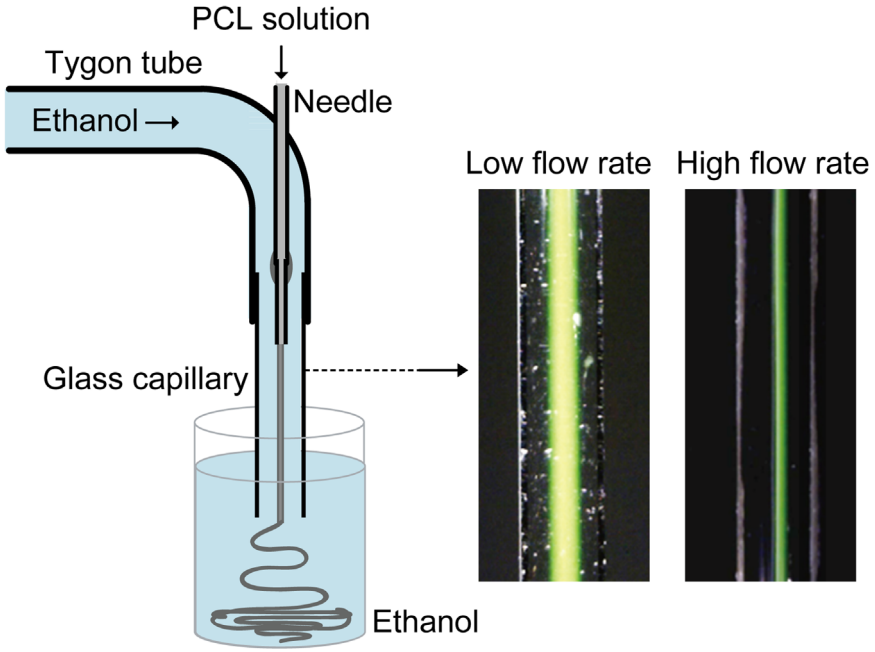

Fig. 1. A schematic diagram of a simple fluidic device for producing a microfibrous matrix and optical images of a glass capillary at low (0.5 mL/min) and high (3.0 mL/min) flow rates of the continuous phase during fabrication.